Amendments to the Specification:

Please add the following paragraph before paragraph **[0001]** (see page 1, lines 8-10) entitled "STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH" and after the Title of the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of, and claims the benefit of priority from, U.S. Patent Application Serial No. 09/158,180, filed September 21, 1998 (now abandoned), the full disclosure of which is incorporated herein by reference in its entirety.

Please replace the text appearing between paragraph **[0044]** (see page 11, lines 7-15) and paragraph **[0045]** (see page 11, lines 23-31) with the following replacement text.

Applicants 250 SREGYTAWFCGTNEDFAKYASNIRKVAADYREKYAFVFLDT 290
(SEQ ID NO: 5 [[3]])

Blunt et al. 250 SREGYTPGSVVLTRTSPSMLQTLERLQLITEKSMPLFSLDT 290
(SEQ ID NO: 4)

Please replace paragraph **[0070]** (see page 19, lines 3-17) with the following replacement paragraph.

In preferred embodiments, the assay reagents use recombinantly produced polyclonal or monoclonal antibodies that bind to the PDI as binding moieties. Recombinant antibodies are typically produced by immunizing an animal with the PDI, obtaining RNA from the spleen or other antibody-expressing tissue of the animal, making cDNA, amplifying the variable domains of the heavy and light immunoglobulin chains, cloning the amplified DNA into a phage display vector, infecting *E. coli*, expressing the phage display library, and selecting those library members that express an antibody that binds to PDI. Methods suitable for carrying out each of these steps are described in, for example U.S. patent no. 6,555,310, issued April 29, 2003

~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~. In preferred embodiments, the antibody or other binding peptides are expressed on the cell surface of a replicable genetic unit, such as a filamentous phage, and especially phage M13, Fd and F1. Most work has inserted libraries encoding polypeptides to be displayed into either gIII or gVIII of these phage, forming a fusion protein which is displayed on the surface of the phage. *See, e.g.*, Dower, WO 91119818; Devlin, WO 91/18989; MacCafferty, WO 92/01047 (gene III); Huse, WO 92/06204; Kang, WO 92/18619 (gene VIII).

Please replace paragraph **[0071]** (see page 19, lines 18-27) with the following replacement paragraph.

In a preferred embodiment, the genes that encode the heavy and light chains of antibodies present in the cDNA library are amplified using a set of primers that can amplify substantially all of the different heavy and light chains. The resulting amplified fragments that result from the amplification step are pooled and subjected to asymmetric PCR so that only one strand (*e.g.*, the antisense strand) is amplified. The single strand products are phosphorylated, annealed to a single-stranded uracil template (e.g., the vector BS45, described in U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159~~, which has coding regions for the constant regions of mouse heavy and light chains), and introduced into a uracil DNA glycosylase$^+$ host cell to enrich for vectors that contain the coding sequences for heavy and light chain variable domains.

Please replace paragraph **[0073]** (see page 20, lines 4-9) with the following replacement paragraph.

In a preferred embodiment, the library is enriched for those phage that display more than one antibody that binds to PDI. Methods and vectors that are useful for this enrichment are described in U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159~~. The

panning can be repeated one or more times to enhance the specificity and sensitivity of the resulting antibodies. Preferably, panning is continued until the percentage of functional positives is at least about 70%, more preferably at least about 80%, and most preferably at least about 90%.

Please replace paragraph [0074] (see page 20, lines 10-17) with the following replacement paragraph.

A recombinant anti-PDI monoclonal antibody can then be selected by amplifying antibody-encoding DNA from individual plaques, cloning the amplified DNA into an expression vector, and expressing the antibody in a suitable host cell (*e.g. E. coli*). The antibodies are then tested for ability to bind PDI. An example of a recombinant monoclonal antibody prepared using this method is the mAb CP.2~~, which was deposited under the Budapest Treaty with the American Type Culture Collection (10801 University Boulevard, Manassas, Va. 20110-2209) on _____; and has been assigned ATCC Accession No. _____~~.

Please replace paragraph [0075] (see page 20, lines 18-26) with the following replacement paragraph.

Recombinant polyclonal antibodies are particularly preferred, in particular because of the various forms of PDI that may be found in clinical samples due to, for example, proteolysis. The diverse fine binding specificity of members of a population of polyclonal antibodies often allows the population to bind to several forms of PDI (*e.g.*, species variants, escape mutant forms, proteolytic fragments) to which a monoclonal reagent may be unable to bind. Methods for producing recombinant polyclonal antibodies are described in ~~co-pending,~~ commonly assigned U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~. Specific methods of producing recombinant polyclonal antibodies that bind to PDI are described in the Examples below.

Please replace paragraph **[0079]** (see page 21, lines 21-28) with the following replacement paragraph.

The capture reagent can be any compound that specifically binds to PDI. Examples of binding moieties that are suitable for use as capture reagents are described above. One example of a suitable capture reagent is the recombinant polyclonal antibody preparation SCPc.4.PC, which was prepared as described in the Examples. ~~Cells that produce these recombinant polyclonal antibodies were deposited under the Budapest Treaty with the American Type Culture Collection (10801 University Boulevard, Manassas, Va. 20110-2209) on _____, and this deposit has been assigned ATCC Accession No. _____.~~

Please replace paragraph **[0117]** (see page 32, line 24 to page 33, line 2) with the following replacement paragraph.

PCR primers were made corresponding to the coding sequence at the 5' and 3' ends of the *C. parvum* PDI, primers B and C, respectively (Table 1). The primers were based on a sequence found in the literature (Blunt *et al.*, *supra.*). In addition, the 5' primer contains 20 base pairs of vector sequence at its 5'-end corresponding to the 3'-end of the pBRnsiH3 vector (described in ~~copending,~~ commonly assigned U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~). The 3' primer contains the 19 base pairs of the tet promoter removed by *Hin*dIII digestion, in addition to 20 base pairs of vector sequence 3' to the HindIII site at its 5' end (*see*, Example 18 of U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~).

Please replace paragraph **[0118]** (see page 33, lines 3-13) with the following replacement paragraph.

The PDI insert was amplified with the primers described above and 1 µl (~50 ng) of *C. parvum* genomic DNA as template per reaction. The amplification (3x 100 µl reactions) was performed using Expand™ DNA polymerase and the reactions pooled and purified as described in Example 19 of U.S. patent no. 6,555,310 ~~application Ser. No. 08/835,159~~. The PDI insert (100 ng) was annealed with the pBRnsiH3 (100 ng) at a 3:1 molar excess of insert to vector, and an aliquot electroporated into 40 µl of electrocompetent *E. coli* strain, DH10B as described in Example 9. The transformed cells were diluted to 1.0 ml with 2xYT broth and 10 µl, 100 µl, and 300 µl plated on LB agar plates supplemented with tetracycline (10 µg/ml) and grown overnight at 37° C. Four colonies were picked into 3 ml 2xYT supplemented tetracycline (10 µg/ml) and grown overnight at 37° C. The following day, glycerol freezer stocks were made for long term storage at -80° C.

Please replace the text appearing between paragraph **[0121]** (see page 34, lines 1-6) and paragraph **[0122]** (see page 34, lines 12-13) with the following replacement text.

Applicants 250 SREGYT**AWFCGTNEDFAKYASNIRKVAADYREKYAFVFLDT** 290
(SEQ ID NO: 5 [[3]])

Blunt et al. 250 SREGYT**PGSVVLTRTSPSMLQTLERLQLITEKSMPLFS**LDT 290
(SEQ ID NO: 4)

Please replace paragraph **[0123]** (see page 34, lines 14-19) with the following replacement paragraph.

The inventors believe that PDI sequence described herein is the correct sequence based on the following: 1) the identical insertion and deletion occurred in four clones from two independent cloning experiments; 2) the frame-shifted sequence described herein shares a greater percent identity ~~identify~~ to protein disulfide isomerase from other organisms than does the same region from the literature. The *Cryptosporidium parvum* protein disulfide isomerase antigen was expressed and purified as described in Example 15.

Please replace paragraph **[0129]** (see page 37, lines 8-15) with the following replacement paragraph.

To amplify substantially all of the H and L chain genes using PCR, primers were chosen that corresponded to substantially all published sequences. Because the nucleotide sequences of the amino terminals of H and L contain considerable diversity, 33 oligonucleotides were synthesized to serve as 5' primers for the H chains, and 29 oligonucleotides were synthesized to serve as 5' primers for the kappa L chains as described in ~~co-pending,~~ commonly assigned U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~. The constant region nucleotide sequences required only one 3' primer each to the H chains and the kappa L chains. *Id.*

Please replace the legend appearing following Table 2 (see page 38, lines 20-22) just prior to paragraph **[0133]** (see page 38, line 24 to page 39, line 9) with the following replacement legend.

Buffer A is 25 mM Tris, 1 mM EDTA, pH 8.0

Buffer B is 25 mM Tris, 1 mM EDTA, 1 M NaCl, pH 8.0
Buffer C is 40 mM [[mm]] phosphoric acid

Please replace paragraph **[0134]** (see page 39, lines 12-25) with the following replacement paragraph.

One ml of *E. coli* CJ236 (BioRAD, Hercules, CA) overnight culture was added to 50 ml 2xYT in a 250 ml baffled shake flask. The culture was grown at 37° C. to OD_{600} = 0.6, inoculated with 10 µl of a 1/100 dilution of BS45 vector phage stock (described in ~~co-pending,~~ commonly assigned U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~) and growth continued for 6 hr. Approximately 40 ml of the culture was centrifuged at 12 krpm for 15 minutes at 4° C. The supernatant (30 ml) was transferred to a fresh centrifuge tube and incubated at room temperature for 15 minutes after the addition of 15 µl of

10 mg/ml RNaseA (Boehringer Mannheim, Indianapolis, IN). The phage were precipitated by the addition of 7.5 ml of 20% polyethylene glycol 8000 (Fisher Scientific, Pittsburgh, PA.)/3.5M ammonium acetate (Sigma Chemical Co., St. Louis, MO.) and incubation on ice for 30 min. The sample was centrifuged at 12 krpm for 15 min at 2-8° C. The supernatant was carefully discarded, and the tube was briefly spun to remove all traces of supernatant. The pellet was resuspended in 400 µl of high salt buffer (300 mM NaCl, 100 mM Tris pH 8.0, 1 mM EDTA), and transferred to a 1.5 ml tube.

Please replace paragraph **[0150]** (see page 45, lines 5-15) with the following replacement paragraph.

Panning was continued by diluting phage samples into panning buffer as described above or by enriching the phage samples by panning using 7F11 magnetic latex (described in Examples 21 and 22 of U.S. patent no. 6,555,310, issued April 29, 2003 ~~application Ser. No. 08/835,159, filed Apr. 4, 1997~~) prior to functional panning (*see*, Example 16 of U.S. patent no. 6,555,310 ~~application Ser. No. 08/835,159~~). The progress of panning was measured by plating aliquots of each latex sample on 100 mm LB agar plates to determine the percentage of kappa positives. The majority of latex from each panning (99%) was plated on 150 mm LB agar plates to amplify the phage binding to the latex (see above). The 100 mm LB agar plates were incubated at 37° C. for 6-7 hr, after which the plates were transferred to room temperature and nitrocellulose filters (pore size 0.45 mm, BA85 Protran, Schleicher and Schuell, Keene, NH) were overlayed onto the plaques. Plates with nitrocellulose filters were incubated overnight at room temperature.

Please replace paragraph **[0151]** (see page 45, lines 16-21) with the following replacement paragraph.

After the overnight incubation, the next round antibody phage was eluted from the 150 mm plates, and the filters were developed with goat anti-mouse kappa alkaline phosphatase as described in Example 13. Individual phage samples having kappa positive percentages of greater than 80% on plaque lifts were pooled. The pooled phage was subcloned into the expression

vector, pBRncoH3. The subcloning was done generally as described in Example 18 of U.S. patent no. 6,555,310 ~~application Ser. No. 08/835,159~~.

Please replace paragraph **[0163]** (see page 49, lines 4-13) with the following replacement paragraph.

Phage samples enriched for binding to crude *Cryptosporidium* antigen as described in Example 14 were pooled using an equal number of phage from each sample. Biotinylated CP.2 monoclonal antibody (12 µl, 10^{-6} M) and soluble crude *Cryptosporidium* antigen (12 µl, about 2 mg/mL) were mixed and incubated for 10 min at room temperature. Twenty µl of CP.2 biotin/antigen was added to the phage sample, and the sample was incubated overnight at 2-8° C. The sample was panned with avidin magnetic latex and plated as described in Example 14. The eluted phage was panned a second time as described using biotinylated CP.2/crude *Cryptosporidium* antigen. The phage eluted after the second round of panning were subcloned as described in Example 18 of U.S. patent no. 6,555,310 ~~application Ser. No. 08/835,159~~. This polyclonal was designated SCPc.4.PC.